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Evidence of astrogliosis in rat hippocampus after D-amphetamine exposure

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Abstract

Introduction: Psychostimulants such as amphetamine (AMPH) induce manic-like symptoms in humans and studies have suggested that bipolar disorder (BD) may be associated to dopamine dysfunction. Glial fibrillary acidic protein (GFAP) up-regulation is considered a marker of astrogliosis, and it has been associated to behavioral sensitization.
Purpose: We aimed to investigate the behavioral effects of acute and chronic AMPH on rat locomotion and assess GFAP levels in rat cortex and hippocampus.
Methods: Rats were administered either acute (single dose) or chronic (seven days) D-amphetamine IP injection. Locomotion was assessed with an open-field test and GFAP immunoquantity was measured using ELISA.
Results: Chronic, but not acute, administration of AMPH increased GFAP levels in rat hippocampus. No differences were observed in rat cortex.
Conclusions: Repeated exposure to AMPH leads to an astroglial response in the hippocampus of rats.

Keywords: Amphetamine; Animal model; Bipolar disorder; Glial fibrillary acidic protein (GFAP); Mania

1. Introduction

Both acute and chronic use of psychostimulants such as amphetamine (AMPH) induce manic-like symptoms in humans (Asghar et al., 2003; Strakowski and Sax, 1998), and AMPH is widely used as an animal model of mania (Cappeliez and Moore, 1990; Niculescu et al., 2000; Machado-Vieira et al., 2004). Recently, a postmortem study demonstrated increased D1 mRNA expression in hippocampal CA2 subregion of bipolar disorder (BD) patients (Pantazopoulos et al., 2004). Moreover, Vogel et al. (2004) reported decreased D3 mRNA levels in peripheral lymphocytes of BD patients. Taken together, these studies suggest that dopaminergic changes may play a role in the pathophysiology of BD. Indeed, D2-blockers are considered first-line agents in the treatment of acute mania (Yatham et al., 2005). AMPH enhances dopamine neurotransmission by reversing dopamine transporters on nerve terminals (Jones et al., 1998), and induces acute and long-term loss of dopamine and serotonin terminals (Davidson et al., 2001). However, the exact mechanisms underlying AMPH-induced neurotoxicity are not fully understood.

Increased reactive oxygen/nitrogen species formation after repeated AMPH administration has been associated with AMPH-related behavioral changes and neurodegeneration (Brown and Yamamoto, 2003; Fukami et al., 2004). Thomas et al. (2004) recently demonstrated that only "neurotoxic" amphetamines lead to increased microglia activation and glial fibrillary acidic protein (GFAP) expression, and "non-neurotoxic" amphetamines did not have this effect. GFAP is the major protein of astrocytic

Abbreviations: AMPH, amphetamine; BD, bipolar disorder; ERK, extracellular signaling-regulated kinase; GFAP, glial fibrillary acidic protein; GSK3-β, glycogen synthase kinase 3-β.

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intermediate filaments, and its up-regulation is considered a marker of astrogliosis and neurotoxicity (Eng et al., 2000; Fatemi et al., 2004). In the present study we aimed to assess the occurrence of astrogliosis, as measured by the immunoquantity of GFAP, in the cerebral cortex and hippocampus of rats after acute and chronic AMPH administration.

2. Methods

2.1. Animals

Adult male Wistar rats (obtained from our breeding colony), weighting 220–310 g, were housed five to a cage maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). Food and water were available ad libitum. All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care.

2.2. Experimental design

We used two experimental groups: acute (single injection) and chronic (one daily injection for 7 days) treatment. In the acute treatment, we administered a single intraperitoneal injection of either saline (control), or 1 mg/kg (AMPH 1), 2 mg/kg (AMPH 2), or 4 mg/kg (AMPH 4) of D-amphetamine (Sigma, USA) ($n=10$ per group). In the chronic treatment, rats

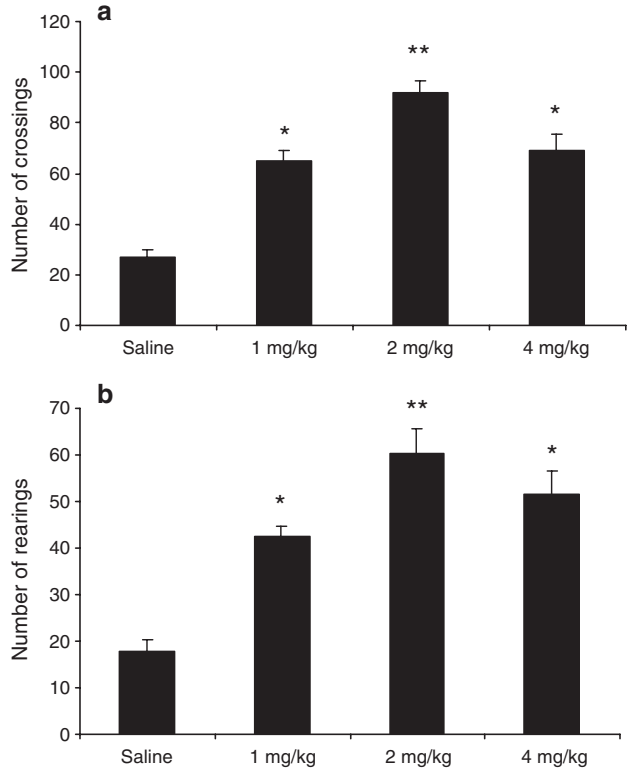

Fig. 1. Numbers of crossings (a) and rearings (b) after acute AMPH treatment (single injection; $n=10$ animals per group). *$p<0.05$ (saline vs. 1 and 4 mg/kg). **$p<0.05$ (2 mg/kg vs. 1 and 4 mg/kg).

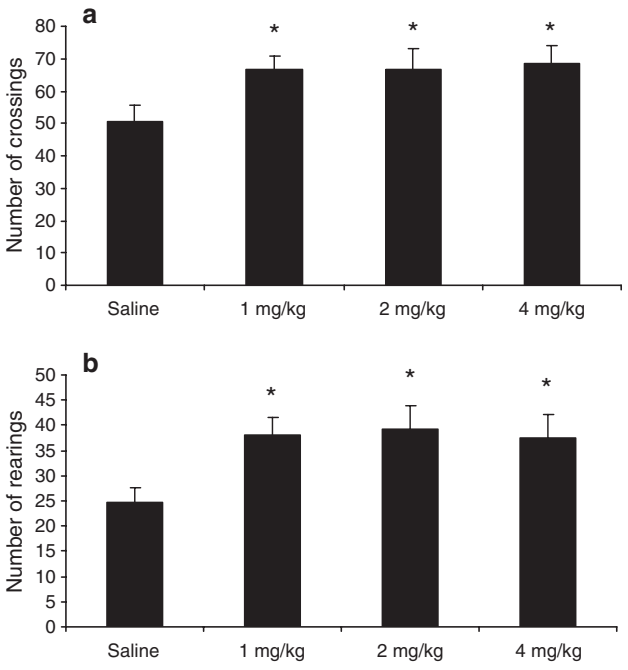

Fig. 2. Numbers of crossings (a) and rearings (b) after chronic AMPH treatment (once daily injection, 7 days; $n=10$ animals per group). *$p<0.05$ (saline vs. 1, 2 and 4 mg/kg).

($n=10$ per group) were administered the same protocol once daily for 7 consecutive days. Locomotor activity was measured 2 h after the last injection of AMPH, and the rats were sacrificed by decapitation right after the behavioral experiment, in order to evaluate the relationship between behavioral response and GFAP levels. Hippocampus and cerebral cortex were dissected, rapidly frozen, and stored at − 80 °C until assayed.

2.3. Locomotor activity

The open-field task was performed in a 40 × 60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min. Crossings of the black lines and rearings performed were counted.

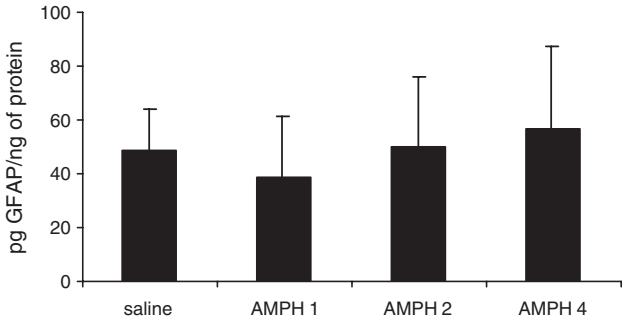

Fig. 3. Levels of GFAP (pg)/protein (ng) after acute AMPH treatment in rat hippocampus (single injection; $n=10$ animals per group).